Labeling maize (Zea mays L.) leaves with 15 NH4+ and monitoring nitrogen incorporation into amino acids by GC/MS analysis

Titre	Labeling maize (Zea mays L.) leaves with 15 NH4+ and monitoring nitrogen incorporation into amino acids by GC/MS analysis
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Résumé en anglais	The human body contains approximately 3.2% nitrogen (N), mainly present as protein and amino acids. Although N exists at a high concentration (78%) in the air, it is not readily available to animals and most plants. Plants are however able to take up both nitrate (NO3−) and ammonium (NH4+) ions from the soil and convert them to amino acids and proteins, which are excellent sources for all animals. Most N is available as the stable isotope 14N, but a second form, 15N, is present in very low concentrations. 15N can be detected in extracts of plants by gas chromatography followed by mass spectrometry (GC/MS). In this protocol, the methods are described for tracing the pathway by which plants are able to take up 15N-labeled nitrate and ammonium and convert them into amino acids and proteins. A protocol for extracting and quantifying amino acids and 15N enrichment in maize (Zea mays L.) leaves labeled with 15NH4+ is described. Following amino acid extraction, purification, and separation by GC/MS, a calculation of the 15N enrichment of each amino acid is carried out on a relative basis to identify any differences in the dynamics of amino acid accumulation. This will allow a study of the impact of genetic modifications or mutations on key reactions involved in primary nitrogen and carbon metabolism.
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